

FIG. 1A

H36.D2.B7 Anti-Tissue Factor Light Chain Variable Region

GACATTAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTACCATCACATGC  
D I Q M T Q S P A S Q S A S L G E S V T I T C  
CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC  
L A S Q T I D T W L A W Y Q Q K P G K S P Q L  
CTGATTATGCTGCCACCAACTTGGCAGATGGGCTCCATCAAGGTTAGTGGCAGTGGATCTGGCACA  
L I Y A A T N L A D G V P S R F S G S G S G T  
AAATTTCTTCAGATCAGCAGCCTACAGGCTGAAGATTTGTAATTATTACTGTCAACAAGTTAC  
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y  
AGTTCTCCATTACGTTGGTCTGGGACCAAGCTGGAGCTGAAA  
S S P F T F G A G T K L E L K

FIG. 1B

H36.D2.B7 Anti-Tissue Factor Heavy Chain Variable Region

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTAAGCCTGGGCTTCAGTGCAGGTATCCTGCAAG  
E I Q L Q Q S G P E L V K P G A S V Q V S C K  
ACTTCTGGTTACTCATTCACTGACTACAACGTGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCTTGAG  
T S G Y S F T D Y N V Y W V R Q S H G K S L E  
TGGATTGGATATATTGATCCTTACAATGGTATTACTATCTACGACCAGAACTCAAGGGCAAGGCCACA  
W I G Y I D P Y N G I T I Y D Q N F K G K A T  
TTGACTGTTGACAAGTCTCCACCACAGCCTTCATGCATCTAACAGCCTGACATCTGACGACTCTGCA  
L T V D K S S T T A F M H L N S L T S D D S A  
GTTTATTCTGTGCAAGAGATGTACTACGGCCCTTGACTTCGGGCCAAGGCACCACCTCACAGTC  
V Y F C A R D V T T A L D F W G Q G T T L T V  
TCCTCA  
S S

\* CDR regions underlined.

Antibody	Apparent $K_a$ , M $^{-1}$	Apparent $K_d$ , M
By ELISA		
D2	$5.2 \times 10^9$	$1.9 \times 10^{-10}$
I47	$6.5 \times 10^9$	$1.5 \times 10^{-10}$
K73	$9.8 \times 10^9$	$1.0 \times 10^{-10}$
K80	$2.3 \times 10^9$	$4.3 \times 10^{-10}$
L102	$2.5 \times 10^9$	$4.0 \times 10^{-10}$
L133	$1.7 \times 10^9$	$5.9 \times 10^{-10}$
By BLACore		
H36	<u><math>3.1 \times 10^{10}</math></u>	<u><math>3.2 \times 10^{-11}</math></u>
I43	$2.3 \times 10^9$	<u><math>4.3 \times 10^{-10}</math></u>
I47	$3.2 \times 10^9$	<u><math>3.1 \times 10^{-10}</math></u>
L133	$4.6 \times 10^9$	<u><math>2.2 \times 10^{-10}</math></u>
M107	$1.1 \times 10^9$	<u><math>9.1 \times 10^{-10}</math></u>

FIG. 2

0590586 "112104"

Antibody Name	% Inhibition	
	Antibody Preincubated with TF/VIIa	
D1		0
D1B		1
H31		4
<u>H36</u>		<u>95</u>
I43		1
J131		7
K80		0
K82		0
K87		1
L97B		7
L101		0
L102		0
L105		0
L133		0
M5		1
M107		34

FIG. 3

0909088-422101

Antibody Name	% Inhibition	
	TF Preincubated with Antibody Prior to Addition of VIIa	TF Preincubated with VIIa Prior to Addition of Antibody
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
I43	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

FIG. 4

101-24566-6

[rhTF], nM	[H36.D2], nM	H36.D2/rhTF Molar Ratio	Clotting Time (seconds)	% Inhibition of rhTF Function
0.0048	0	0	102.3	0
	1.61	335.4	114.3	31.3
	3.23	670.8	121.3	45.8
0.023	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
	6.45	280.4	99.6	73.9
0.092	0	0	49.3	0
	3.23	35.1	65.8	65.2
	6.45	70.1	88.5	90.2
	12.90	140.2	113.3	95.7
0.46	0	0	32.6	0
	6.45	14.0	52.7	82.4
	12.90	28.0	80.2	96.7
	32.30	70.2	117.9	99.3
2.30	0	0	23.9	0
	16.10	7.0	47.1	94.4
	32.30	14.0	95.2	99.7
	64.50	28.0	115.3	99.9
11.52	0	0	22.2	0
	16.10	1.4	30.2	93.4
	32.30	2.8	46.0	98.8
	64.50	5.6	87.6	99.9
	161.30	14.0	114.0	100.0

FIG. 5

09260586-122101

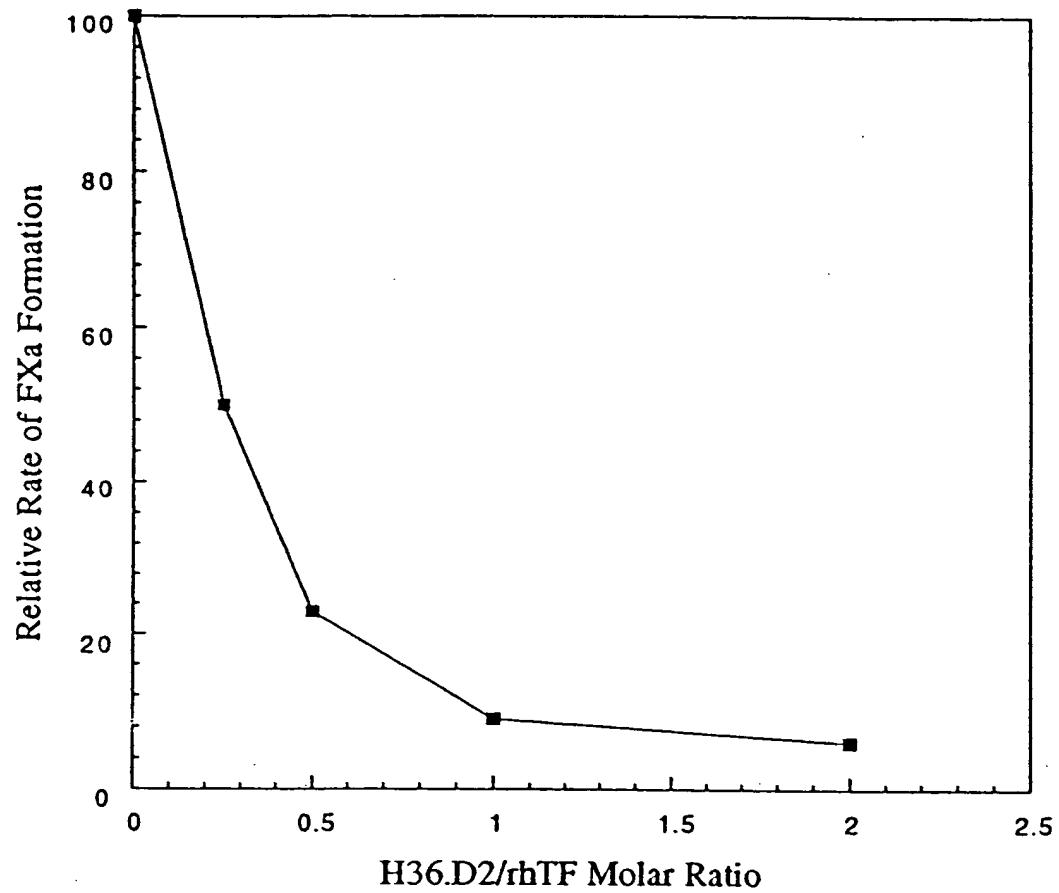


FIG. 6A

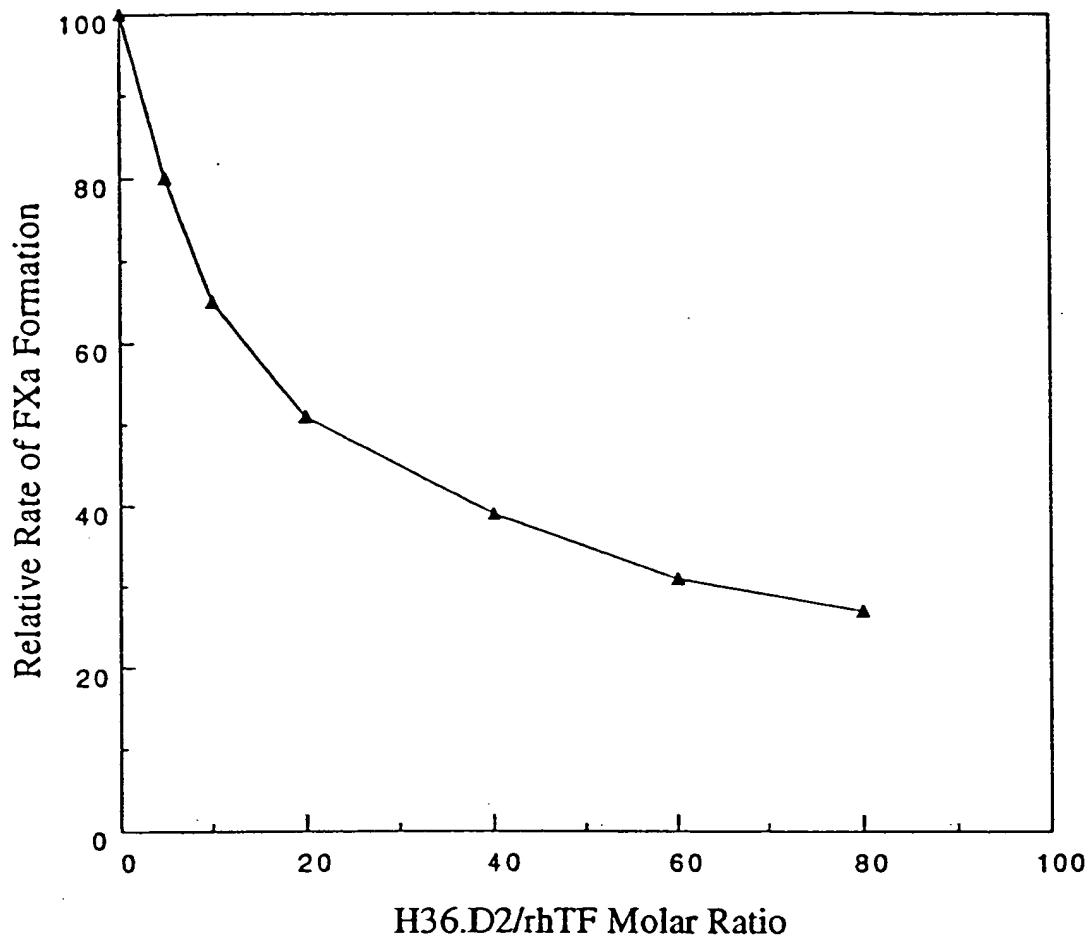


FIG. 6B

H36.D2 Concentration (ng)	<u>% Inhibition</u> Cells (TF/FVII) and H36.D2 preincubated prior to FX addition	<u>% Inhibition</u> FX and H36.D2 are added simultaneously to Cells (TF/FVII)
0	0	0
50	88	nd
100	92	nd
200	97	nd
800	nd	76
1600	nd	78
3200	nd	92

FIG. 7

09090506-112101

2 $\mu$ l, 60ng

Native  
Urea

1 $\mu$ l, 30ng

Native  
Urea

0.5 $\mu$ l, 15ng

Native  
Urea

8M urea  
5mM DTT w

FIG. 8A

0999586 112101  
T01

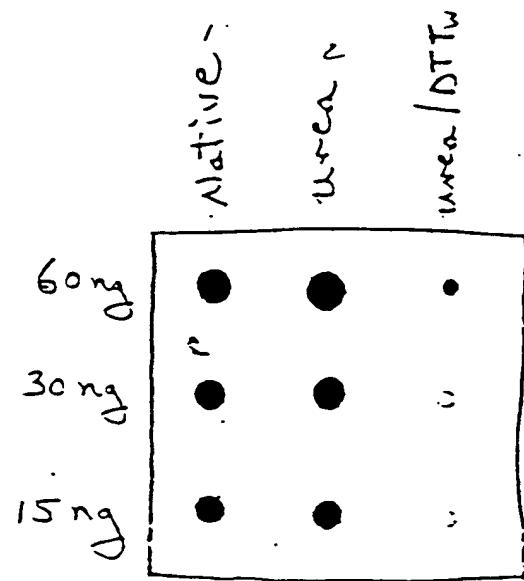


FIG. 8B

**Figure A. Human IgG1-cH36 HC Variable Region Cloning and Expression Vector**

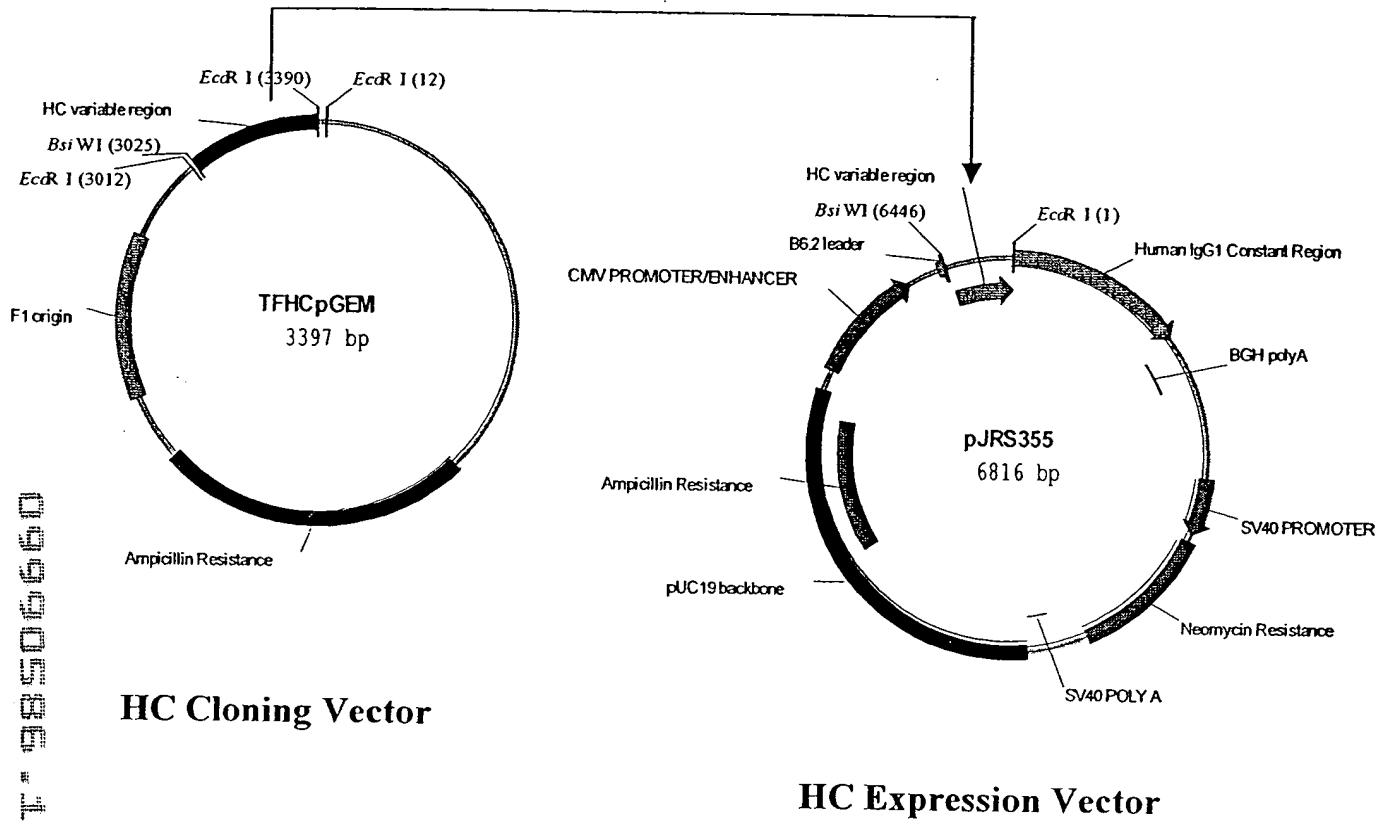


Fig. 9A

Fig. 9B

**Figure B. Human IgG4-cH36 HC Variable Region Cloning and Expression Vector**

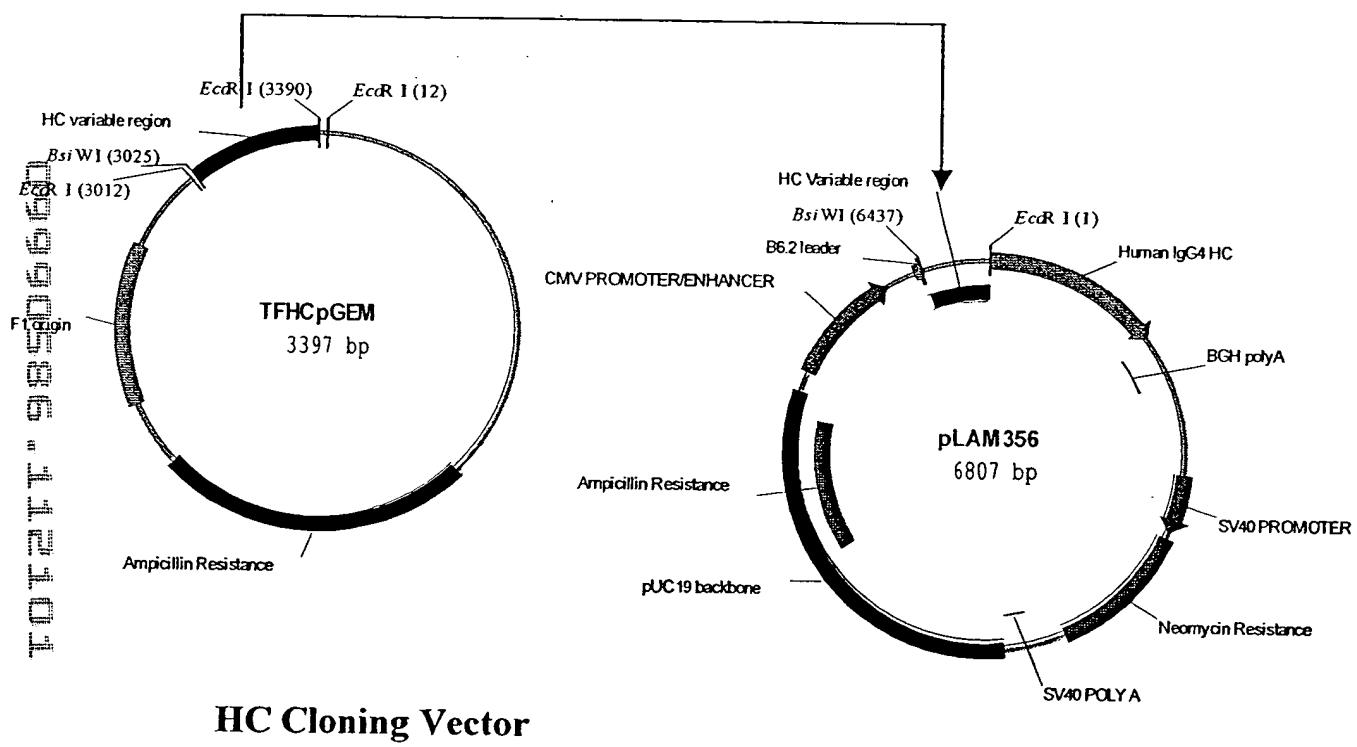


Fig. 9C

**HC Expression Vector**

Fig. 9D

**Figure C. cH36 LC Variable Region Cloning and Expression Vector**

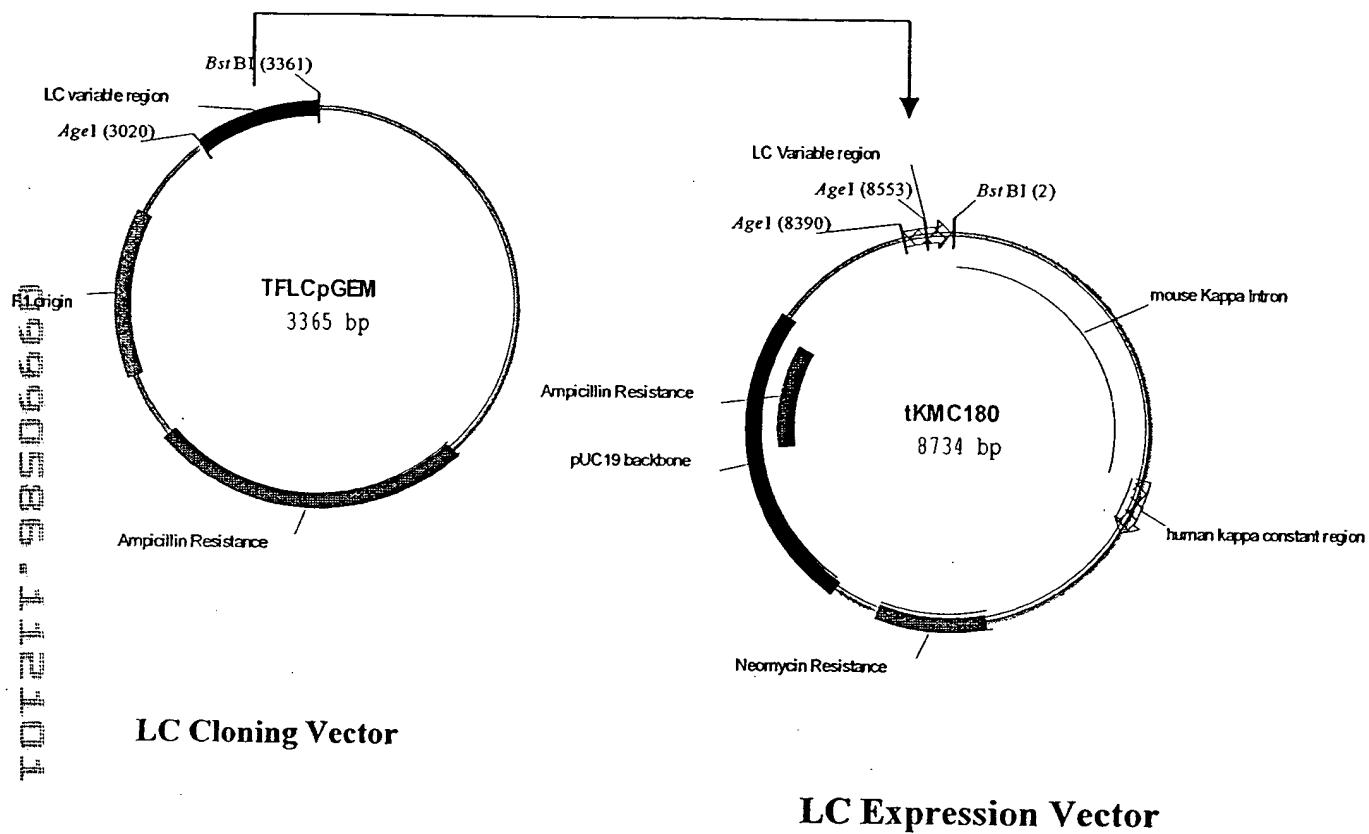
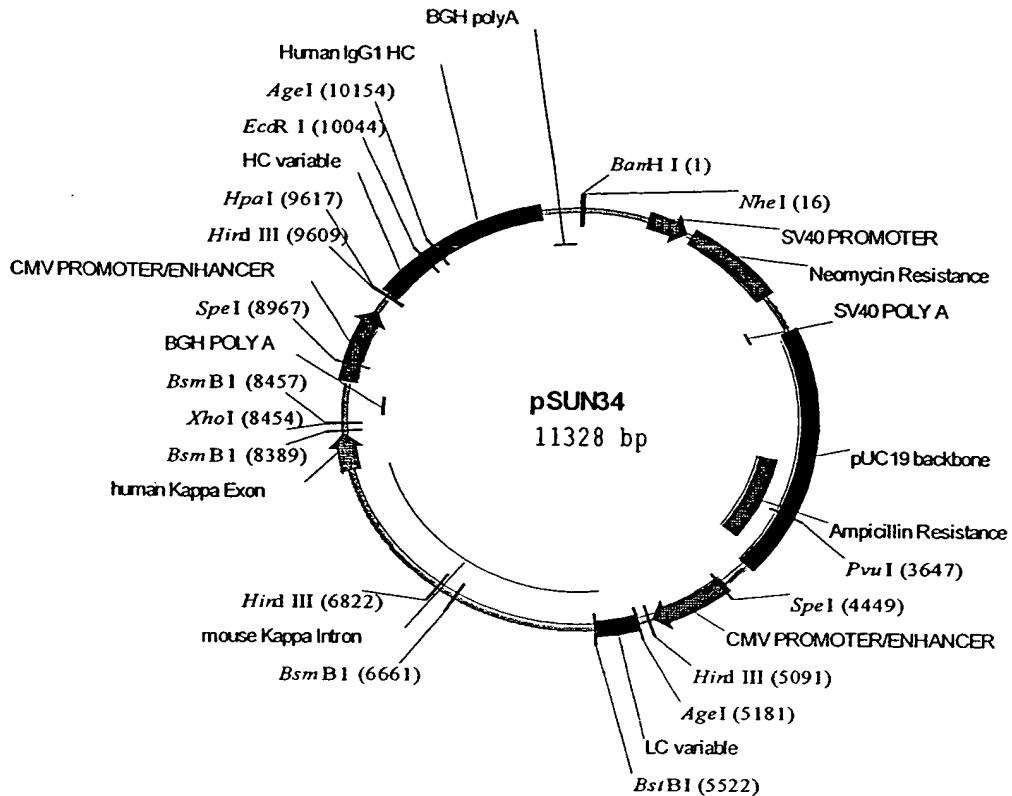


Fig. 10A

Fig. 10B



**Figure D. Plasmid Map of Humanized Anti-TF IgG1 Antibody Expression Vector**

Fig. 11

0200596-110101

# Humanization of Anti-Tissue Factor Antibody CH36

## Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions

### Light Chain (LC) FR Sequences

FR1 (23 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (10 AA)	Names
10	20	35	47 57 60 70	98 107
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFSKISSLQ <b>AEDFVNYYC</b>	<b>EGAGTKLEIK</b>	CH36-LC
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFSKISSLQ <b>AEDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-03
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFSKISSLQ <b>AEDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-04
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFSKISSLQ <b>AEDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-05
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFSKISSLQ <b>AEDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-06
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFSKISSLQ <b>AEDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-07
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFS <b>EF</b> ISSLQ <b>EDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-08
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFS <b>EF</b> ISSLQ <b>EDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-09
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFS <b>EF</b> ISSLQ <b>EDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-10
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFS <b>EF</b> ISSLQ <b>EDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-11
DIQMTQSPASQASASLGESVTITC	WYQQKPGKSPOLIY	<b>GVPSRFSGSGSGT</b> KFS <b>EF</b> ISSLQ <b>EDFVNYYC</b>	<b>EGAGTKLEIK</b>	LC-12

### Light Chain CDR Sequences of CH36

CDR1 (11 AA)	CDR2 (7 AA)	CDR3 (9 AA)
24 IASQTRIDTWA	34 AATNLA	56 Q2VYSSPFT
		89 97

Fig. 12B

Fig. 12D

Fig. 12C

Fig. 12A

Fig. 12A

# Sequences of Partially and Fully Humanized Heavy Chain (HC) Variable Regions

## Heavy Chain (HC) FR Sequences

FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (11 AA)	Names
1 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	CH36-HC
2 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-01
3 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-02
4 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-03
5 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-04
6 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-05
7 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-06
8 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-07
9 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-08
10 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-08R1
11 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-11
12 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-12
13 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-09
14 10 20 29 36 44 67 75 85 95 107 117	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	WVRQSHGKSLIEWIG KATLTVDKSSTTAFMHNSLTSDDSAVYFCAR WGGQGTITLVSS	HC-10

## Heavy Chain CDR Sequences

CDR1 (5 AA)	CDR2 (17 AA)	CDR3 (8AA)	Names
31 35 50	Y I D P Y N G I T I Y D Q N F K G	99 66	CH36
31 35 50	Y I D P Y N G I T I Y D Q N Y K G	99 66	HC-08
31 35 50	Y I D P Y N G I T I Y D Q N Y K G	99 106	
31 35 50	Y I D P Y N G I T I Y D Q N Y K G	106	

Fig. 13B

Fig. 13C

Fig. 13D

## hOAT (IgG1) Constant regions sequences

Sequences of IC constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWVKVDNALQSGNSQESVTEQDSKDSTYSSLSSSTLTLSKADYEKH

Fig. 14A

KVYACEVTHQGLSSPVTKSFNRGEC

Sequences of HC constant:

EFASTKGPSVFPPLAPSSKSTSGGTAAALGCLVKDVFPEPVTVSWNSGALTSGVHTFPAVVLQSSGLYSLSVVTPSSSLGTQTYIC

Fig. 14B

NVNHKPSNTKVDKKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV

HNAAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL

VKGFYPSDIAVEWESNGQQPENNYKTTPPVLDGSSFFLYSKLTVDKSRWQQGNVFSCSVVMHEALHNHYTQKSLSSLSPGK

## hFAT (IgG4) constant region sequences

### Sequences of LC Constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVVDNALQSGNSQESVTEQDSKDSTYSLSSSTLTSKADYEK  
HKVYACEVTHQGLSSSPVTKSFNRGEC

### Sequences of HC constant:

EFASTKGPSVFPPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY  
TCNVDHKPSNTKVDKRVESKYGPPCPCSPAPEFLGGPSVFLFPPKPKDILMISRTPEVTCVVVDVSSQEDPEVQFNWYVVDGV  
EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSVNKGPSSEKTISKAKGQPREPQVYTLPPSQEEMTKKNQVSL  
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSSLSSLGK